Evolution of Vertebrate Olfactory Systems

Abstract
The general features of the olfactory system are remarkably consistent across vertebrates. A phylogenetic analysis of central olfactory projections indicates that at least three distinct olfactory subsystems may be broadly present in vertebrates and that a fourth, the accessory olfactory or vomeronasal system, arose in tetrapods. The origin and function of the vomeronasal system have been the subject of much controversy, but some conclusions can be drawn. The vomeronasal system did not arise as an adaptation to terrestrial life, as indicated by the presence of a vomeronasal system in modern aquatic amphibians and the increasing paleontological evidence that the last common ancestor of amphibians and amniotes was aquatic. The vomeronasal system is involved in both foraging and reproductive behaviors in reptiles and has been shown to be involved in some pheromonaally mediated behaviors in mammals. However, among mammals, some pheromonal responses are not mediated by the vomeronasal system, and the possible involvement of the vomeronasal system in other types of behaviors has not yet been investigated. Thus, the relative functions of the olfactory and vomeronasal systems of tetrapods remain unclear. Other hypotheses that features of the olfactory system are specialized for aquatic chemoreception or for pheromone detection are similarly insupportable. For example, the suggestion that members of the olfactory receptor family can be separated into two groups that function for transduction of air-borne or water-borne odorants is contradicted by the presence of both groups in aquatic amphibians and by a phylogenetic analysis of the sequences for these genes. Interestingly, the putative odorant receptors from the vomeronasal epithelium share little sequence similarity with those from the olfactory epithelium, indicating that these receptors may have been independently co-opted from the larger family of seven transmembrane domain receptors for use in odor transduction. A phylogenetic analysis of the distribution of olfactory receptor cell types indicates that microvillar olfactory receptor cells are widespread among vertebrates and are not restricted to aquatic animals or to the vomeronasal epithelium of tetrapods. Previous suggestions that all microvillar receptor cells are specialized for the detection of pheromones are not tenable. Attempts to recognize features of the olfactory system that are common to all vertebrates and might be specialized for the detection of pheromones vs. more general odorants, or for the detection of water-borne vs. air-borne odorants, are not supported by current evidence.
Introduction

The origin of vertebrate sensory systems is a mysterious event about which relatively little is known because of the large morphological gap between vertebrates and other chordates. In contrast to this major leap, most sensory systems, including olfaction, have undergone fairly modest changes during the course of vertebrate phylogeny. In this paper, I will illustrate the distribution of features of the olfactory system across vertebrates, with a view to constructing hypotheses concerning the extent and nature of changes that have occurred. My intention is to be provocative, to point out areas where mistakes have been made or large questions remain unanswered, as well as to speculate about patterns and processes in the evolution of the vertebrate olfactory system.

The topics covered in this paper proceed from fairly minute and peripheral features, the odorant receptor proteins and the extensions of the receptor cells on which they are located, through a review of the organization of the olfactory bulb and its central projections, to a discussion of the possible functions of the olfactory subsystems. Recurring themes include questions concerning differences in organization among olfactory systems that function in aquatic or terrestrial habitats and questions concerning the possible existence of a subsystem that is specialized for detection of pheromones, compounds involved in communication among conspecifics, analogous to the specialized pheromone-processing systems that are widespread among insects.

In addition to the main olfactory system, tetrapods possess an accessory olfactory system, the receptor neurons of which are sequestered in the vomeronasal organ, also known as the organ of Jacobson. For ease of expression, I will refer to this system as the accessory olfactory or vomeronasal system and the more phylogenetically widespread system simply as the olfactory system. Thus, although it is common to use the term 'main olfactory bulb' to refer to the primary target of the olfactory receptor cells in tetrapods, I will refer to this structure simply as the olfactory bulb to avoid confusion when making comparisons with non-tetrapods. Finally, I will not discuss the terminal nerve in this paper. Although this enigmatic anterior cranial nerve has been suggested to serve a sensory function [Dems-ki and Northcutt, 1983], more recent evidence indicates that this nerve functions instead as a neuromodulatory system [Oka, 1992; Wirsig-Wiechmann, 1993; Wirsig-Wiechmann and Jennes, 1993].

Putative Odorant Receptors

Only a few years ago, Buck and Axel [1991] isolated from the olfactory epithelium of rats members of a large group of genes encoding proteins that are part of the seven transmembrane domain receptor superfamily. Similar genes have now been found in diverse vertebrates, including mice [Nef et al., 1992], humans [Ben-Arie et al., 1994], chickens [Leibovici et al., 1996], catfish [Ngai et al., 1993], and zebrafish [Weth et al., 1996]. Although the proteins have not yet been demonstrated to function as odorant receptors, the size and diversity of the gene family, as well as its relatively restricted expression in olfactory receptor neurons, are consistent with the hypothesis that these are odorant receptors. The same proteins also appear to be involved in axonal guidance [Mombaerts et al., 1996].

Freitag and his colleagues recently described the sequences and expression patterns of 19 members of the odorant receptor gene family in Xenopus [Freitag et al., 1995]. The olfactory epithelium of Xenopus is found in two separate chambers of the nasal cavity, the more dorsal of which is open only when the animal has its head above water and the more ventral of which is open only when the head is underwater. Based on the expression patterns of these genes, as well as on a distance analysis of the sequences of these and other members of the putative olfactory receptor gene family, the authors suggested that these genes may be grouped into two classes; those that are found in terrestrial animals, such as rats and mice, as well as the genes from the dorsal olfactory epithelium in Xenopus; and those that are found in aquatic animals, such as catfish, as well as the genes from Xenopus' ventral olfactory epithelium. Thus, the two classes of receptor proteins were suggested to function to detect air-borne and water-borne odorants, respectively. However, more recent findings contradict this hypothesis. Hansen and her colleagues have examined the expression pattern of the olfactory receptor genes during development and find that members of both classes are found in the olfactory epithelium in larval Xenopus, which are fully aquatic and possess a single olfactory chamber [Hansen et al., 1996]. Furthermore, Zhou et al. [1996] have cloned 12 members of the same family from mudpuppies (Necturus maculosus), which are neotenic aquatic salamanders. Both maximum likelihood and parsimony analyses of all available sequences of these putative olfactory receptor genes indicate that the gene family cannot be divided easily into two groups, and that some mudpuppy receptor genes are most similar to those from mammals, while others are more similar to those from catfish. Thus, it appears that we cannot yet draw conclusions con-
cerning functional or evolutionary groups within this
subfamily of receptors.

Members of the seven transmembrane domain receptor
superfamily have also been found in the vomeronasal ep-
thelium of rats [Dulac and Axel, 1995]. Again, although
functional expression of these genes has not yet been
achieved, the size of the gene family and the restriction
of its expression to vomeronasal receptor cells indicate that
these may be odorant receptors. These receptor genes bear
very little sequence similarity to those found in the ol-
factory epithelium, which raises the interesting possibility
these two subfamilies were coopted independently from
the larger family of seven transmembrane domain receptors
to function as odorant receptors.

Morphology of Receptor Cells

Olfactory receptor cells can be divided into two cate-
gories, ciliated and microvillar, based on the membrane spe-
cializations present on the apical surface of the cell. Al-
though new evidence indicates that numerous subgroups can
be distinguished within these broad categories [Morita and
Finger, 1996], most published papers simply describe receptor
cells as being ciliated or microvillar, and I will use these
simplistic categories in the following analysis. Because I
have already examined the phylogenetic distribution of re-
ceptor cell types in the olfactory and vomeronasal epithelia
[Eisthen, 1992], I will not review the data here, although I
will discuss some of the conclusions that can be drawn.

Both ciliated and microvillar olfactory receptor cell
types may be derived from a single common ancestral type.
Most cells contain only two centrioles, which anchor the
ends of the chromosomes during cell division and remain in
the cell thereafter. In cells that will develop cilia, however,
these centrioles are duplicated several times, and the result-
ing centrioles anchor the bases of cilia. Curiously, micro-
villar olfactory and vomeronasal receptor cells frequently
contain multiple centrioles, suggesting that these cells might
be derived from ciliated cells [Eisthen, 1992]. In addition,
Morita and Finger have recently described a class of cells in
the olfactory epithelium of catfish that terminate in mi-
crovilli but contain partial cilia buried within the dendrite
[Morita and Finger, 1996]. Both of these observations are
consistent with the idea that an evolutionary alteration in
the development of ciliated cells gave rise to microvillar
receptor cells and suggest that receptor cell morphology
might be evolutionarily labile.

Unlike the morphologically diverse olfactory receptor
cells, vomeronasal receptor neurons invariably terminate in
microvilli [Eisthen, 1992]. A prevailing notion within the
chemosensory community is that most vertebrates possess
ciliated olfactory receptor cells and that microvillar receptor
cells are an aberration possessed by only a few groups of
animals. Nevertheless, the presence of microvillar olfactory
receptor cells is widespread among vertebrates, as illus-
trated in figure 1. An examination of the phylogenetic dis-
tribution of these receptor cell types leads to three conclu-
sions. (1) Both receptor cell types are sufficiently widely
distributed that the ancestral condition for vertebrates may
be the presence of both ciliated and microvillar olfactory
receptor cells. (2) Microvillar olfactory receptor cells are
present in some terrestrial animals, such as postmetamor-
phic tiger salamanders [Eisthen, 1992], contradicting the
common assertion that microvillar olfactory receptor cells
occur only in aquatic animals. (3) The phylogenetically
widespread presence of microvillar receptor cells in the ol-
factory epithelium, as well as the absence of ciliated recep-
tor cells in cartilaginous fishes, indicates that microvillar
cells are not uniquely specialized for detection of phero-
mones [e.g., Dulka, 1993].

Fig. 1. Phylogenetic distribution of receptor cell types in the ol-
factory epithelium. All taxa that have been examined at the electron
microscopic level are included. Boldface letters indicate cell types:
C=ciliated receptor cell; M=microvillar receptor cell; X=receptor
cell with both cilia and microvilli; B=brush cell, a morphologically
distinct category of microvillar receptor cell. Given the distribution
of receptor cell types, it is equally parsimonious to postulate that the
ancestral condition is the presence of both ciliated and microvillar re-
ceptor cells or that the ancestral condition is the presence of only cili-
ated receptor cells.
Organization of the Olfactory Bulbs

The classical literature on the comparative anatomy of the olfactory bulb of nonmammals has been masterfully reviewed by Nieuwenhuys [1967]. I will not repeat most of the details here but will examine the larger trends in the evolution of olfactory bulb organization. The textbook model of the organization of a vertebrate olfactory bulb is that of a rodent, which possesses seven clearly defined layers: (1) an external fiber layer consisting of the axons of the olfactory receptor cells; (2) a layer of glomeruli, the round bundles of neuropil in which the receptor cell axons form synapses with cells of the olfactory bulb, which also contains periglomerular interneurons; (3) the external plexiform layer containing the somata of small output cells, called tufted cells; (4) a layer containing the cell bodies of mitral cells, the large output cells of the olfactory bulb; (5) an internal plexiform layer; (6) a deep layer of granule cells, small interneurons with processes directed into the more external layers of the bulb; and (7) an ependymal layer. In mammals, each mitral cell has a single dendrite that projects to a glomerulus and several ‘basal dendrites’ that extend horizontally into the fiber layer above. Of course, this organization contains some elements that are common to all vertebrates, but it represents a derived condition. As indicated in figure 2, clearly defined laminar boundaries, plexiform layers, and tufted cells are all innovations that are unique to tetrapods. Based on the descriptions provided by Nieuwenhuys, the primitive condition of vertebrate olfactory bulbs would appear to be the presence of crude glomeruli, mitral cells, and granule cells arranged in layers with indistinct boundaries. In general, mitral cells project to multiple glomeruli, and granule cells have axons that exit the olfactory bulb.

This view of olfactory bulb organization is undoubtedly too simplistic, and different cell types have probably been lumped into a few broad categories. In hagfish and lampreys, neurons with somata located in the glomerular layer have been interpreted as externally displaced mitral cells, but may instead be periglomerular cells. If the latter is correct, then the presence of periglomerular cells, which clearly occur in the olfactory bulbs of ray-finned fishes and tetrapods, should be considered to be the ancestral condition for vertebrates. Most authors refer to any large output cells as mitral cells, although the mitre-shaped cell body that gives these cells their name is only obvious in tetrapods; perhaps one or more different types of output cells are present in non-tetrapods. In his study of the olfactory bulb of teleosts, Catois [1902] referred to large output cells with an elongated, horizontally oriented cell body as ‘fusiform’ cells. Morphologically similar cells are apparent in illustrations of the olfactory bulb of sturgeons [Johnston, 1898] and may be present in other vertebrates as well. Additional types of output cells have been recognized in other teleosts [see, e.g., Holmgren, 1922; Alonso et al., 1987], although distribution of these cell types across teleosts has not been established. Finally, many authors appear to classify all cells with small somata that are located deep in the olfactory bulb as granule cells. The similarity of these cells across vertebrates is questionable, particularly given that the granule cells of tetrapods possess short axons, as in tiger salamanders [Herrick, 1948], or lack axons completely, as in mammals. Furthermore, multiple categories of granule cells have been recognized in most vertebrates that have been examined, but the phylogenetic distribution of these cell types is difficult to establish [Nieuwenhuys, 1967].
Thorough morphological and histochemical studies are sorely needed to establish the identities of the cells present in the olfactory bulbs of nonmammalian vertebrates.

The olfactory and accessory olfactory bulbs of tetrapods are organized similarly, except that the laminae of the accessory olfactory bulb have less distinct boundaries, the plexiform layers are thinner, tufted cells are not present, and the mitral cells project to multiple glomeruli, even in mammals [reviewed in Lohman and Lammers, 1967; Nieuwenhuys, 1967]. It is interesting to note that the accessory olfactory bulb possesses the same suite of characters that are primitive for the olfactory bulb of bony fishes and tetrapods, although, given its central projections, the accessory olfactory bulb is clearly the phylogenetically newer system. This observation suggests that the accessory olfactory bulb is essentially a duplicate of the primitive olfactory bulb and that the structure of the olfactory bulb diverged after the accessory olfactory bulb arose. How would such a duplication occur? The development of the olfactory bulb of frogs and rats is induced by an interaction between the ingrowing axons of the olfactory receptor cells and the telencephalic primordium [Burr, 1916; Graziaedi and Monti Graziaedi, 1992; Gong and Shipley, 1995]. If the development of the accessory olfactory bulb is the result of a similar inductive process, then a simple evolutionary change in the developmental timing or location of a subset of neurons in the nasal placode could have resulted in the formation of a separate vomeronasal epithelium and accessory olfactory bulb.

Central Projections of the Olfactory Systems

Much research into the location and extent of olfactory bulb projections in vertebrates has been stimulated by Edinger’s [1904] suggestion that the telencephalon was originally an olfactory structure that was invaded by other sensory systems over the course of vertebrate evolution. I will not describe the projections of the olfactory system in all animals for which data are available; instead, I will describe the projections in one species in each vertebrate class for which detailed data have been obtained using modern neuroanatomical methods.

The olfactory projections in Pacific hagfish (Eiptatetus stouti) have been investigated using injections of diI or horseradish peroxidase into different portions of the olfactory bulb [Wicht and Northcutt, 1993]. Fibers extending from the lateral olfactory bulb project widely in the forebrain, and the targets include the striatum, all layers of the pallium, and a portion of the central prosencephalic nucleus ipsilaterally to the injection site; a subset of fibers extends to similar targets contralaterally to the injection site. A short fiber tract projects from the medial olfactory bulb to the ipsilateral septum and contralateral olfactory bulb. Finally, a group of fibers extends from the ventrolateral portion of the olfactory bulb to terminate diffusely along a path extending into the diencephalon, including the hypothalamus and dorsal thalamus. Given its location and targets, this tract may be a homologue of the extrabulbar olfactory pathway that has been described in amphibians and actinopterygians by Hofmann and Meyer and by Szabo and his colleagues [Hofmann and Meyer, 1989; Szabo et al., 1991; Hofmann and Meyer, 1992, 1995].

Northcutt and Puzdrowski [1988] used injections of horseradish peroxidase into various regions of the olfactory bulb to uncover four major projections into the forebrain of silver lampreys (Ichthyomyzon unicuspis). A lateral olfactory tract projects ipsilaterally throughout the lateral pallium and either to or through the dorsal and medial pallia, as well as to the posterior tuberculum and hypothalamus; some fibers project contralaterally to a dorsal portion of the lateral pallium, septum, striatum, and the posterior tuberculum and hypothalamus. A second group of fibers, which may comprise a homologue of the medial olfactory tract, originates from the ventrolateral olfactory bulb and projects to the ipsilateral septum, preoptic area, and possibly to the rostral portion of the striatum. A third group of fibers extends through the ventromedial olfactory bulb to the striatum and preoptic regions, as well as to the hypothalamus and throughout the posterior tuberculum. Although the authors originally suggested that this latter projection constituted a portion of the terminal nerve, recent immunohistological evidence suggests that lampreys lack a terminal nerve [Eislen and Northcutt, 1996] and that these fibers may instead constitute an extrabulbar olfactory pathway. Finally, silver lampreys possess a fourth and possibly uniquely derived olfactory projection to the adjacent dorso-medial neuropil, a fibrous region that spans the length of the olfactory bulb, and to glomeruli in the contralateral olfactory bulb.

In one of few published studies of olfactory projections in a cartilaginous fish, Smeets [1983] stained degenerating fibers following transection of the olfactory peduncle in the spotted dogfish (Scyliorhinus canicula). A lateral pathway was found to project ipsilaterally to the area retrobulbaris, striatum, lateral pallium, and area superficialis basalis, similar to the lateral olfactory tract that has been described in Ginglymostoma [Ebbesson and Heimer, 1970]. Smeets also described a widely projecting medial pathway, which he interpreted as a medial olfactory tract. Others have suggested

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that this pattern of degeneration may be the result of a lesion of the lateral pallium, which lies in close proximity to the peduncle, and that sharks may lack a medial olfactory tract [e.g., Northcutt, 1995].

The forebrain of ray-finned fishes is everted and contains many discrete cell groups, the homologies of which are difficult to establish. Because connectivity is a main criterion for homology, any attempt to compare the similarity of olfactory projection patterns between teleosts and other vertebrates can become circular, and independent evidence, such as histochemical data, is necessary to corroborate hypotheses of homology. Among nonteleost ray-finned fishes, the olfactory projections have been most carefully examined in the bichir, Polypterus palma [Braford and Northcutt, 1974; von Bartheld and Meyer, 1986]. In Polypterus, a lateral olfactory tract projects largely to the pallial area P3 and may contain some contralaterally projecting fibers, and a medial tract projects ipsilaterally to the pallial area P1 and bilaterally to ventral telencephalic regions, with some fibers extending as far caudal as the hypothalamus. Based on its position and its massive input from the olfactory bulb, P1 is generally considered to be the homologue of the lateral pallium [Northcutt and Davis, 1983; von Bartheld and Meyer, 1986], and topological considerations have led to the suggestion that P3 is the homologue of the medial pallium/hippocampus [Northcutt and Davis, 1983; von Bartheld and Meyer, 1986; Braford, 1995]. The central projections of the olfactory bulb have been described for several teleosts and have been examined in some detail in goldfish (Carassius auratus) using horseradish peroxidase [von Bartheld et al., 1984; Levine and Dethier, 1985]. The lateral olfactory tract of goldfish projects mainly to a dorsolateral pallial area, as well as projecting bilaterally to nucleus taeniae and the hypothalamic region. The medial olfactory tract projects bilaterally to the ventral forebrain areas Vs, VI, and Vv, which may be equivalent to part of the septum; to Vd, which may be the equivalent of part of the striatum; and to DI and the preoptic area, with some fibers terminating as far caudally as the hypothalamus. Although the existence of an extrabulbar olfactory pathway has not yet been demonstrated in goldfish, the lectin binding properties of the pathway were used to investigate projections in the spiny eel, Macragnostus aculeatus, and in two perciforms, Mogurnda mogurnda and Hemichromis lifalili [Hofmann and Meyer, 1995]. In the first two species, fibers bypassing the olfactory bulb were found to project to Vv and the preoptic area, whereas in the third the fibers terminate in Vv and Vs.

Among amphibians, the olfactory projections have been examined in tiger salamanders [Kokoros and Northcutt, 1977] and in several anurans, including three species of ranid frogs [Scala, 1972; Northcutt and Royce, 1975; Kemali and Guglielmotti, 1987; Scala et al., 1991]. In ranids, the lateral olfactory tract projects ipsilaterally to the lateral pallium, dorsal striatum, cortical amygdaloid nucleus, and a region interpreted as either a portion of the dorsal pallium [Northcutt and Royce, 1975] or a dorsal portion of the lateral pallium [Scala et al., 1991]. A medial olfactory tract projects to the postolfactory eminence and medial pallium as well as to the lateral and medial septal nuclei. Fibers of the medial and lateral olfactory tracts project in combination to the contralateral cortical amygdaloid nucleus and lateral pallium. Fibers of the accessory olfactory tract project bilaterally to both the cortical amygdaloid nucleus and the medial amygdala sensu Scala et al. [1991]. The extrabulbar olfactory pathway of Xenopus has been examined in detail by Hofmann and Meyer, who have found that fibers originating in the olfactory epithelium bypass the olfactory bulb and terminate in the ipsilateral preoptic area and bilaterally in the hypothalamus [Hofmann and Meyer, 1991a, b, 1992].

The projections of the olfactory bulb have been examined in relatively few reptiles. Although older studies had indicated the presence of three major olfactory projections, consisting of lateral, medial, and intermediate olfactory tracts, comparisons with projections in other tetrapods indicate that the "intermediate" olfactory tract can often be understood as a subdivision of one of the other tracts. Among snakes, the most detailed study is that of Halpern [1976], who used degenerating fiber stains to examine the olfactory projections in garter snakes (Thamnophis sirtalis and T. radix). The lateral olfactory tract of these animals projects ipsilaterally to the anterior olfactory nucleus and lateral portion of the olfactory tubercle and bilaterally to the lateral pallium. An intermediate olfactory tract projects along the ventromedial edge of the lateral olfactory tract to the medial portion of the anterior olfactory nucleus and to the rostral portion of the lateral pallium. Halpern suggested that this tract might be equivalent to the medial olfactory tract of other tetrapods, but the identity of its targets suggests that this tract may instead constitute a portion of the lateral olfactory tract. The accessory olfactory tract projects to the ipsilateral nucleus sphericus, a relatively large forebrain structure that probably constitutes a portion of the amygdala but which has no certain homologues in other tetrapods [Bruce and Neary, 1995]. Using both degeneration techniques and lectin labeling, the olfactory projections in the lizard Gekko gecko have been examined in some detail [Lohman et al., 1988]. A lateral olfactory tract projects to the anterior olfactory nucleus, olfactory tubercule, external amygdaloid nucleus, and lateral cortex. An intermedi-
ate olfactory tract was observed to project to the septum; this tract may be the homologue of the medial olfactory tract. Further caudally, these two tracts combine to project bilaterally to the lateral cortex and to the external and central amygdaloid nuclei. Finally, an accessory olfactory tract projects ipsilaterally to the external and central amygdaloid nuclei, bed nucleus of the stria terminalis, and nucleus sphericus. Projections of the olfactory bulbs have also been traced in pigeons (Columba livia) and red-eared turtles (Pseudemys scripta) using an autoradiographic technique [Reiner and Karten, 1985]. In both animals, a lateral olfactory tract has a massive bilateral projection to a lateral cortical area, and a medial olfactory tract projects ipsilaterally to the septum and to a medial cortical area that may be the homologue of the medial pallium/hippocampus of other vertebrates. In both animals, an intermediate olfactory tract that may be a subdivision of the lateral tract projects to the olfactory tubercle, as well as to the medial striatum in pigeons and to the basal amygdaloid nucleus in turtles. In contrast to these similarities between pigeons and turtles, the extent of the olfactory projection to the amygdala was found to differ dramatically. In pigeons, olfactory input is restricted to nucleus taeniae of the archistriatum, which is a portion of the amygdala, whereas in turtles a large olfactory projection was observed along the entire pial surface of the amygdala. Although the authors compare olfactory projections in turtles and crocodiles and suggest that the olfactory input to the amygdala may be more extensive in animals that lack a vomeronasal system, in fact the vomeronasal system is present in turtles and absent in birds [Parsons, 1967]. Perhaps the distinction between the olfactory and accessory olfactory bulbs of turtles is less clear than in other reptiles, and the authors inadvertently included the accessory olfactory bulb in some injections, in which case the large ‘olfactory’ projection to the amygdala observed in this study may also comprise an accessory olfactory projection.

The projections of the olfactory and accessory olfactory bulbs of mammals have been reviewed many times [e.g., Halpern, 1987; Wysocki and Meredith, 1987], and I will not provide elaborate details here. To my knowledge, separate lateral and medial olfactory tracts have not been described in mammals, although most authors appear to have been interested in delineating the differences in projections of the olfactory and accessory olfactory bulbs, rather than seeking evidence for segregated olfactory bulb projections. In rats, projections of the olfactory bulb have been traced to the ipsilateral anterior olfactory nucleus, anterior hippocampus, olfactory tubercle, and pyriform and entorhinal cortices as well as to the anterior cortical amygdala, posterior amygdala, and the nucleus of the lateral olfactory tract [Scalia and Winans, 1975]. Bilateral projections of the olfactory bulb, present in most other vertebrates, appear to have been lost in mammals [Skeen et al., 1984]. Fibers from the accessory olfactory bulb project ipsilaterally to the bed nucleus of the stria terminalis, portions of the posterior cortical and medial nuclei of the amygdala, and to the nucleus of the accessory olfactory tract [Scalia and Winans, 1975]. In addition, mammals may possess an extrabulbar olfactory pathway. Monti Grazidei [1993] has described an olfactory projection to a portion of the hypothalamus, the fibers of which are immunoreactive for olfactory marker protein, indicating that they arise from the olfactory epithelium, but lack immunoreactivity to luteinizing hormone releasing hormone, indicating that they are not part of the terminal nerve system. The relative position, target, and immunocytochemical profile of these fibers are similar to those that characterize the extrabulbar olfactory pathway in other vertebrates.

The peripheral portion of the vomeronasal system in mammals contains two subdivisions. In rats and opossums, vomeronasal receptor neurons contain one of two unique G proteins, and the cell bodies of the neurons containing Go, are located deeper in the epithelium than are the cell bodies of the neurons containing Go2 [Halpern et al., 1995; Berghard and Buck, 1996]. The axons of these two classes of receptor neurons project to discrete terminal fields within the accessory olfactory bulb, with those of the Go-containing neurons projecting to the posterior portion and those of the Go2-containing neurons projecting to the anterior portion [Halpern et al., 1995]. The anterior and posterior subdivisions of the accessory olfactory bulb can also be differentially labeled with lectins and with antibodies to olfactory marker protein, glycolipids, and other neuronal components [Imamura et al., 1985; Mori et al., 1987; Shnayder et al., 1993; Schwarting et al., 1994; Shapiro et al., 1995]. Taken together, these data suggest that the vomeronasal system of mammals contains at least two subdivisions that may process information differently or may respond to different types of stimuli. Perhaps the separate portions of the accessory olfactory bulb also give rise to two separate accessory olfactory tracts, but experiments to test this idea have not yet been carried out.

Although the telencephalic projections of the olfactory bulb may be more restricted in gnathostomes [Northcutt and Puzdrowski, 1988; Wicht and Northcutt, 1993], broad similarities can be detected across vertebrate classes. As illustrated in figure 3, the available data suggest that the ancestral condition for vertebrates is the presence of lateral, medial, and extrabulbar olfactory pathways. Across verte-
many vertebrates may also possess an extrabulbar olfactory pathway consisting of axons of primary olfactory receptor neurons that bypass the olfactory bulb and project to the basal forebrain, particularly the preoptic and hypothalamic regions.

It is perplexing to note that the lateral olfactory tract of Polypterus projects to a suspected homologue of the medial pallium/hippocampus and that the medial tract projects to a suspected homologue of the lateral pallium, which is the reverse of the general pattern in vertebrates. Perhaps the apparent medial and lateral tracts have been reversed in Polypterus in conjunction with the eversion of the telencephalon. If this is the case, then we would expect that the tracts in other ray-finned fishes, such as goldfish, would be similarly reversed. Although the medial olfactory tract of goldfish projects bilaterally to dorsolateral pallial areas, as is typical of the lateral olfactory tract of other vertebrates, this medial tract also projects to ventral areas that are suspected homologues of portions of the septum and striatum, which is typical of the medial tract of other vertebrates. Thus, the relationships between the medial and lateral olfactory tracts in ray-finned fishes and other vertebrates remain ambiguous.

**Functions of the Subsystems**

In the preceding section, I argued that vertebrates generally have at least three central olfactory projections and that tetrapods have at least four, including the projections of the vomeronasal system via the accessory olfactory tract. Perhaps the olfactory system extracts biologically meaningful components of the signal and processes these components independently, as seems to be the case in the visual system [Schneider, 1969]. Do the olfactory subsystems carry different types of information or function differently?

The possible segregation of functions in the central olfactory projections has not been explored in most animals. Perhaps the most compelling demonstration of functional differences between olfactory projections comes from studies of the medial and lateral olfactory tracts in goldfish, which are ideal subjects for addressing such questions, because the long olfactory tracts that connect the peripherally located olfactory bulbs to the rest of the forebrain can readily be accessed for electrophysiological recording, electrical stimulation, and lesioning. Based on a series of such experiments, it appears that the lateral olfactory tract carries information related to foraging behavior, whereas the medial tract is involved in pheromonal mediation of courtship and spawning behavior [reviewed in Kyle et al., 1987;
Dulka, 1993]. Electrical stimulation experiments with cod (Gadus morhua) have produced similar results [Doving and Selset, 1980]. It should be noted that these studies do not discriminate between the function of the medial olfactory tract per se and the extrabulbar olfactory pathway, which courses through the ventral portion of the medial olfactory tract; however, a clear segregation of function between the medial and lateral projections has been established for goldfish and may be applicable to teleost fishes in general. The possibility that these subsystems may be involved in similar functions in other vertebrates has not yet been determined. Nevertheless, given that tetrapods in general also have segregated medial and lateral olfactory tracts, it is clear that the medial olfactory tract of goldfish is not homologous with the vomeronasal pathway of tetrapods, as has been suggested [e.g., Dulka, 1993].

The olfactory subsystem that has been the subject of most investigation is the vomeronasal system. Noting that only tetrapods possess both olfactory and vomeronasal systems, Broman [1920] suggested that the vomeronasal system in tetrapods is homologous with the nasal chemosensory system of fishes and that the olfactory system arose later as an adaptation to terrestrial life. This must be incorrect, for, given the similar projections of the olfactory bulb in fishes and tetrapods, the vomeronasal system must be the evolutionarily newer system. Bertmar later inverted Broman’s hypothesis, suggesting that the vomeronasal system arose in tetrapods as an adaptation to terrestrial life [Bertmar, 1981]. This hypothesis also seems to be incorrect. First, the vomeronasal system is generally present throughout the lifecycle in both metamorphosing and fully aquatic salamanders, caecilians, and frogs [reviewed in Schmidt and Roth, 1990; Schmidt and Wake, 1990; Eisthen et al., 1994]. If the vomeronasal system arose as an adaptation to terrestrial life, then its presence in larval and aquatic amphibians could only be due to a heterochronic change that has resulted in the early development of the system, which is theoretically possible [Gould, 1977] but has not been demonstrated to occur. Second, the presence of the vomeronasal system in both amphibians and amniotes indicates that the system must have been present in the last common ancestor of these two groups, which was probably aquatic [Panchen, 1991; Lebedev and Coates, 1995]. Indeed, many features that are unique to tetrapods have been interpreted as adaptations to terrestrial life, and such hypotheses have come under increasing scrutiny as paleontologists have found that tetrapods possess many unique features that seem to have arisen together but independent of the transition to life on land [Carroll, 1995; Ahlberg et al., 1996; Clack, 1997].

Despite decades of investigation, the relative functions of the vomeronasal and olfactory systems remain obscure. Among rodents, the vomeronasal system is clearly involved in mediating both behavioral and physiological responses to pheromones [reviewed by Wysocki, 1979; Halpern, 1987; Wysocki and Meredith, 1987]. For example, male mice experience testosterone surges in response to chemical signals from female conspecifs [Macrides et al., 1979], an effect mediated by the vomeronasal system [Wysocki et al., 1983], and sexually inexperienced male mice with vomeronasal organ lesions exhibit almost no mating behavior [Bean and Wysocki, 1985]. Female mice attain puberty at a younger age when exposed to chemical stimuli from adult male conspecifs [reviewed in Vandenberg, 1983] and abort early-stage pregnancies when exposed to chemical stimuli from unfamiliar males [Parks and Bruce, 1962]. Both of these effects are mediated by the vomeronasal and not olfactory system [Kaneko et al., 1980; Lloyd-Thomas and Keverne, 1982].

Based on findings such as these, many researchers have now over-generalized the separation of function of the olfactory and vomeronasal systems, ascribing all pheromone effects to mediation by the vomeronasal system and assuming that the only function of the vomeronasal system is to mediate responses to pheromones [e.g., see reviews by Dulka, 1993; Liman, 1996]. In fact, some pheromone effects are mediated by the olfactory system and not the vomeronasal system. For example, estrus female pigs adopt a stereotyped mating posture when exposed to a compound in the saliva of boars; this behavior can be mediated by the olfactory system and does not require an intact vomeronasal system [Dorries et al., 1997]. Chemical stimuli from conspecific males result in release of luteinizing hormone and subsequent ovulation in anestrous female sheep, and this release occurs even in animals in which vomeronasal input has been eliminated [Cohen-Tannoudji et al., 1989]. In addition, the vomeronasal system may serve other functions besides the mediation of pheromone responses. In squamate reptiles, the vomeronasal system plays a major role in prey-trailing and feeding [Halpern and Frumin, 1979; Kubie and Halpern, 1979] as well as in mating behavior [Kubie et al., 1978]. Among mammals, the possibility that the vomeronasal system is involved in foraging or other nonreproductive behaviors has not been investigated, nor have studies been conducted to determine whether vomeronasal receptor cells respond to compounds other than pheromones. The relative roles of the olfactory and vomeronasal systems are therefore equivocal, and it is incorrect to assume that the vomeronasal system is involved only in mediating pheromone responses or that
binding proteins, G-proteins, or receptors that are localized to the vomeronasal organ are necessarily involved in pheromone transduction [e.g., Khew-Goodall et al., 1991; Dulac and Axel, 1995; Berghard and Buck, 1996]. Perhaps part of the difficulty in determining the relative roles of these systems is that we are comparing the function of one subsystem, the vomeronasal or accessory olfactory system, to the function of the rest of the olfactory system, which consists of several subsystems that may have different functions. If this is correct, the ‘dual olfactory hypothesis’ [Scalia and Winans, 1975], in which the separate projections of the olfactory and vomeronasal systems were suggested to be involved in separate behavioral and physiological functions, should be extended to a consideration of the possible separation of function in the lateral, medial, extra-
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